PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P758PC00	FOR FURTHER ACTION See Form PCT/IPEA/416	
International application No. PCT/DK2004/000407	International filing date *(day/month/year)* 10.06.2004	Priority date *(day/month/year)* 10.06.2003
International Patent Classification (IPC) or national classification and IPC C07K16/14, A61K39/395, C07K14/38, C12N15/11, C12N5/10, A61K38/00, G01N33/50		
Applicant ACE BIOSCIENCES A/S et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 8 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 8 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
05.09.2005	18.10.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Weikl, M Telephone No. +49 89 2399-7518

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/DK2004/000407

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-74 as originally filed

Sequence listings part of the description, Pages

1-37 as originally filed

Claims, Numbers

1-51 received on 07.09.2005 with letter of 05.09.2005

Drawings, Sheets

1/29-29/29 as originally filed

☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/DK2004/000407

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 48 and 50 (partially); 31 (completely)

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. 31 are so unclear that no meaningful opinion could be formed *(specify)*:

see separate sheet

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 48 and 50 (partially)

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form ☐ has not been furnished

☐ does not comply with the standard

the computer readable form ☐ has not been furnished

☐ does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No:
PCT/DK2004/000407

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-30,32-34,37-51
	No:	Claims	35,36
Inventive step (IS)	Yes:	Claims	1-30,32-34,37-51
	No:	Claims	35,36
Industrial applicability (IA)	Yes:	Claims	1-30,32-51
	No:	Claims	none

2. Citations and explanations (Rule 70.7):

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this report has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed

   ☒ filed together with the international application in computer readable form

   ☐ furnished subsequently to this Authority for the purposes of search and/or examination

   ☐ received by this Authority as an amendment on

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional observations, if necessary:

Re Item III.

1. For the present application a partial search report under Article 17(2)(a) PCT in conjunction with Articles 5 and 6 PCT has been issued. The claims or parts of claims relating to inventions on which no international search report has been established (i.e. *current* claims 48 and 50 (original claims 57 and 59) insofar as they relate to other indicator moieties than antibodies) are thus not subject of this international preliminary examination (Rule 66.1(e) PCT).

2. Claim 31 (dependent on claim 30) relates to a fragment comprising an epitope of SEQ ID NO 36 namely one or more of the residues set forth in SEQ ID NO 37 *or a variant of said fragment.*
SEQ ID No 37 itself has been identified as an epitope of SEQ ID NO 36 (having 8 residues). A fragment containing **only one** or **several** of these residues does not constitute an epitope any more.
The unclarity created by this inconsistency becomes even more pronounced when one bears in mind that SEQ ID NO 37 itself contains two variable residues (Xaa) and that claim 31 goes on to claim *variants of said fragments.*
The scope of this claim is thus so unclear that no meaningful opinion can be formed on its novelty, inventive step or industrial application.

Re Item V.

1. Subject-matter of the invention

The present application relates to secreted antigens of *Aspergillus fumigatus.* A cell surface exposed protein encoding an isopropylmalate dehydrogenase B (IMDH B) is shown to be one of the major antigens recognized by *Aspergillus fumigatus* antibodies. Addition of antibodies raised against the recombinant protein or addition of the recombinant protein itself are shown to reduce adhesion of *Aspergillus* conidia to A549 pneumocytes. In addition, IMDH B antibodies reduced *Aspergillus* conidia germination.

2. Documents:

The following documents are referred to in this communication:

D1: DATABASE UniProt [Online] 30 May 2000 (2000-05-30), "3-isopropylmalate dehydrogenase B (EC 1.1.1.85) (Beta-IPM dehydrogenase B) (IMDH B) (3-IPM-DH B)." XP002307500 retrieved from EBI accession no. UNIPROT:LE3B_ASPNG Database accession no. LE3B_ASPNG

D2: DATABASE Geneseq [Online] 24 December 1998 (1998-12-24), "Aspergillus fumigatus protein 3." XP002307501 retrieved from EBI accession no. GSN:AAW69392 Database accession no. AAW69392

D3: BOUCHARA J P ET AL: "Adhesion and pathogenicity in Aspergillus infections" MEDECINE ET MALADIES INFECTIEUSES 1999 FRANCE, vol. 29, no. 11, 1999, pages 705-711, XP002307496 ISSN: 0399-077X

D4: HANSEN MIA YOUNG ET AL: "Allergens in Aspergillus fumigatus: I. Characterization of two different allergen extracts and evaluation of their stability and the importance of carbohydrate for IgE binding" ALLERGY (COPENHAGEN), vol. 49, no. 4, 1994, pages 235-241, XP009040600 ISSN: 0105-4538

D5: REIJULA K E ET AL: "MONOCLONAL ANTIBODIES BIND IDENTICALLY TO BOTH SPORES AND HYPHAE OF ASPERGILLUS-FUMIGATUS" CLINICAL AND EXPERIMENTAL ALLERGY, vol. 22, no. 5, 1992, pages 547-553, XP001184000 ISSN: 0954-7894

3. Novelty (Article 33(2) PCT)

Claims 35 and 36 relate to polypeptide variants of SEQ ID No 36 with a sequence identity of at least 75%. The variants are claimed as part of pharmaceutical compositions or for use as a medicament.
The protein disclosed by D1 exhibits 79% sequence identity with SEQ ID No 36 of the present application. The formulation of this protein within a pharmaceutical composition or its intended use for medicine *in general* can not serve to render the claimed protein novel over the prior art D1 (note that principally every protein will

have *at least some* medical relevance).
Therefore, claims 35 and 36 are not novel in the sense of Article 33(2) PCT.

Re Item VIII.

Sufficiency of Disclosure (Article 5 PCT)

1. Claim 2 relates to antibodies with defined dissociation constants, claims 5 and 6 relate to antibodies which are able to reduce the adhesion or germination of *Aspergillus fumigatus* conidia up to 60%.

However, the application does not teach how to make such antibodies. From the figures (Fig 10 and Fig 13) it becomes apparent, that conventionally made antibodies are not able to achieve such levels of reduction of adhesion or germination.

Furthermore, dissociation constants of less than 10^{-15}M as in claim 2 seem exceptionally high for antibody-antigen associations.

It seems that special procedures have to be employed in order to obtain the claimed antibodies. Thus, the subject-matter of claims 2, 5 and 6 can not be regarded as sufficiently disclosed.

2. Claim 19 relates to an antibody which is capable of binding SEQ ID No 36 and an extracellular homologue of SEQ ID No 36.
From the prior art (D1 and D2) it seems however, that the structural homologues of SEQ ID NO 36 are mainly intracellular. Construction of the claimed antibody thus seems to require undue experimentation from the skilled person.

The same applies to the subject-matter of claims 20 and 21.

Amended claims filed in response to Written Opinion – September 2005

1. An isolated antibody capable of binding the polypeptide of SEQ ID NO:36.

2. The antibody of claim 1, wherein the antibody binds said polypeptide with a dissociation constant of less than 10^{-7}M, e.g. less than 5 X 10^{-8}M, such as less than 10^{-8}M, e.g. less than 5 X 10^{-9}M, such as less than 10^{-9}M, e.g. less than 5 X 10^{-10}M, such as less than 10^{-10}M, e.g. less than 5 X 10^{-11}M, such as less than 10^{-11}M, e.g. less than 5 X 10^{-12}M, such as less than 10^{-12}M, e.g. less than 5 X 10^{-13}M, such as less than 10^{-13}M, e.g. less than 5 X 10^{-14}M, such as less than 10^{-14}M, e.g. less than 5 X 10^{-15}M, or less than 10^{-15}M.

3. The antibody of any of the preceding claims, wherein the antibody is selected from the group consisting of IgG, IgA, IgE, IgM and IgD, wherein IgG preferably is IgG1.

4. The antibody of any of the preceding claims, wherein the antibody is capable of binding an intact Aspergillus fumigatus cell.

5. The antibody of any of the preceding claims, wherein the antibody, or at least an Fab fragment thereof, is capable of reducing the adhesion of Aspergillus fumigatus conidia to lung epithelia in an in vitro assay, preferably reducing said adhesion with at least 20%, e.g. at least 40%, or at least 60%.

6. The antibody of any of the preceding claims, wherein the antibody or at least an Fab fragment thereof, is capable of reducing the germination of Aspergillus fumigatus conidia in an in vitro assay, preferably reducing said adhesion with at least 20%, e.g. at least 40%, or at least 60%.

7. The antibody of any of claims 1-6, wherein the antibody is polyclonal.

8. The antibody of any of claims 1-6, wherein the antibody is monoclonal.

9. The antibody of claim 8, wherein the antibody is a chimeric, human or humanised antibody.

10. The antibody of claim 8, wherein the antibody is a human antibody.

11. The antibody of any of the preceding claims, wherein the antibody is purified.

12. The antibody of any of the preceding claims, wherein the antibody is conjugated to a therapeutic moiety, such as a toxin or a fungicidal agent, or coupled to a detectable substance, such as a radioactive material.

13. The antibody of any of the preceding claims, wherein the antibody is further capable of binding a homologous polypeptide, wherein the homologous polypeptide has a sequence identity of 39% or more, such as 42% or more, e.g. 48% or more, such as 68% or more, e.g. 80% or more, such as 90% or more to the polypeptide of SEQ ID NO:36.

14. The antibody of claim 13, wherein said homologous polypeptide originates from
- an Aspergillus species, such as Aspergillus fumigatus, Aspergillus nidulans, Aspergillus niger, or Aspergillus oryzea,
- Neurospora crassa,
- Saccharomyces cerevisiae,
- a Candida species such as Candida albicans,
- a Coccidioides species, such as Coccidioides posadasii, or Coccidioides immitis,
- a Cryptococcus species, such as Cryptococcus neoformans var. neoformans,
- a Fusarium species,
- a Pneumocystis species,
- a Penicillium species,
or
- Histoplasma capsulatum.

15. The antibody of claim 14, wherein said homologous polypeptide originates from
- an Aspergillus species, such as Aspergillus fumigatus, Aspergillus nidulans, Aspergillus niger or Aspergillus oryzea,
- Candida albicans,
- Coccidioides posadasii,

or

- Cryptococcus neoformans var. neoformans.

16. The antibody of claim 15, wherein said homologous polypeptide originates from an Aspergillus species, such as Aspergillus fumigatus, Aspergillus nidulans, Aspergillus niger or Aspergillus oryzea,

17. The antibody of claim 16, wherein said homologous polypeptide originates from Aspergillus fumigatus.

18. The antibody of claim 17, wherein the said homologous polypeptide is the polypeptide of SEQ ID NO:41.

19. The antibody of any of claims 13-18, wherein said homologous polypeptide is extracellular.

20. The antibody of any of the preceding claims, wherein the antibody further is capable of binding an intact cell of any one or more of

- an Aspergillus species other than Aspergillus fumigatus, such as Aspergillus nidulans, Aspergillus niger, or Aspergillus oryzea,
- Neurospora crassa,
- Saccharomyces cerevisiae,
- a Candida species such as Candida albicans,
- a Coccidioides species, such as Coccidioides posadasii, or Coccidioides immitis,
- a Cryptococcus species, such as Cryptococcus neoformans var. neoformans,
- a Fusarium species,
- a Pneumocystis species,
- a Penicillium species,

or

- Histoplasma capsulatum.

21. The antibody of any of claims 1-11, wherein the antibody is not capable of binding an intact cell of any of

- Neurospora crassa,

- Saccharomyces cerevisiae,
- Candida albicans,
- Coccidioides posadasii, or Coccidioides immitis,
- Cryptococcus neoformans var. neoformans,
or
- Histoplasma capsulatum.

22. The antibody of any of the preceding claims, wherein the antibody is capable of binding an epitope which comprises one or more of the residues of a region of SEQ ID NO:36 selected from the group consisting of: Ser67- Leu71, Ala74-Trp80, Ser191-Arg205, Leu268-Leu273, His292-Pro296, Glu355-Ile360, Asp193-Glu209, Asp193-Ala199, Ile15-Val19, Val75-Trp80, Pro11-Glu18 and the region defined by SEQ ID NO:37, preferably an epitope which is entirely consisting of residues comprised within said region.

23. A pharmaceutical composition comprising an antibody as defined in any of claims 1-22 and a pharmaceutically-acceptable carrier.

24. An antibody as defined in any of claims 1-22 or a composition as defined in claim 23 for use as a medicament.

25. Use of an antibody as defined in any of claims 1-22 or a composition as defined in claim 23 for the manufacture of a medicament for the treatment or prevention of fungal infections.

26. Use of claim 25, wherein the medicament is a medicament for the treatment or prevention of Aspergillus infections, preferably Aspergillus fumigatus infections.

27. Use of claim 25, wherein the medicament is a medicament for the treatment or prevention of a fungal disease selected from the group consisting of: invasive aspergillosis, aspergilloma, and allergic aspergillosis, such as allergic bronchopulmonary aspergillosis.

28. Use of claim 25, wherein the medicament is for use in combination therapy with other antifungal therapy.

29. A composition comprising one or more Aspergillus fumigatus polypeptides selected from the group consisting of: a polypeptide comprising SEQ ID NO:36, a fragment of SEQ ID NO:36 comprising an epitope, and a variant of SEQ ID NO:36 having at least 85% sequence identity to SEQ ID NO:36.

30. An Aspergillus fumigatus polypeptide selected from the group consisting of: a polypeptide comprising SEQ ID NO:36, a fragment of SEQ ID NO:36 comprising an epitope, and a variant of SEQ ID NO:36 having at least 85% sequence identity to SEQ ID NO:36.

31. The polypeptide of claim 30, wherein the polypeptide is a fragment comprising one or more residues of the amino-acid sequences set forth in SEQ ID NO: 37, or a variant of said fragment.

32. A polynucleotide encoding a polypeptide as defined in claim 30 or 31.

33. An expression vector comprising a polynucleotide as defined in claim 32.

34. A host cell transformed or transfected with a polynucleotide as defined in claim 32 and/or an expression vector as defined in claim 33.

35. A pharmaceutical composition comprising

- a polypeptide selected from the group consisting of: a polypeptide comprising SEQ ID NO:36, a fragment of SEQ ID NO:36 comprising an epitope, and a variant of SEQ ID NO:36 having at least 75% sequence identity to SEQ ID NO:36 or a polynucleotide encoding such a polypeptide, and
- a pharmaceutically-acceptable carrier.

36. A polypeptide selected from the group consisting of: a polypeptide comprising SEQ ID NO:36, a fragment of SEQ ID NO:36 comprising an epitope, and a variant of SEQ ID NO:36 having at least 75% sequence identity to SEQ ID NO:36, or a polynucleotide encoding such a polypeptide, for use as a medicament.

37. Use of a polypeptide selected from the group consisting of: a polypeptide comprising SEQ ID NO:36, a fragment of SEQ ID NO:36 comprising an epitope, and a variant of SEQ ID NO:36 having at least 75% sequence identity to SEQ ID NO:36, or a polynucleotide encoding such a polypeptide, for the manufacture of a medicament for the immunisation of a mammal against fungal infections.

38. The use of claim 37, wherein said mammal is a human being.

39. A method for raising specific antibodies to the polypeptide set forth in SEQ ID NO:36 in a non-human mammal comprising the steps of
a. providing a polypeptide as defined in claim 30 or 31, or a cell expressing such a polypeptides,
b. introducing a composition comprising said polypeptide or said cell into said animal,
c. raising antibodies in said animal, and
d. isolating and optionally purifying the antibodies.

40. The method of claim 39, wherein the raising of antibodies is done in a transgenic animal which is capable of producing human antibodies.

41. A method for identifying a binding partner of the polypeptide of SEQ ID NO:36, comprising the steps of
a. providing a polypeptide as defined in any of claims 30 or 31,
b. contacting said polypeptide with a putative binding partner, and
c. determining whether said putative binding partner is capable of binding to said polypeptide.

42. The method of claim 41, wherein the putative binding partner is a host-derived molecule.

43. The method of any of claims 41-42, wherein said method is repeated for a plurality of putative binding partners.

44. A method for identifying a compound with antifungal activity comprising the steps of

a. providing a sensitised cell which has a reduced level of the polypeptide of SEQ ID NO:36 and
b. determining the sensitivity of said cell to a putative antifungal compound, for instance by a growth assay.

45. A method for identifying an inhibitor of the extracellular *Aspergillus* polypeptide set forth in SEQ ID NO:36, comprising the steps of
a. providing two cells which differ in the level of the polypeptide set forth in SEQ ID NO:36,
b. determining the sensitivity of said cells to a putative inhibitor, for instance by a growth assay, and
c. determining whether said two cells are differently affected by the presence of said putative inhibitor.

46. The method of claim 45, wherein the two cells differ in the copy number of said polypeptide.

47. The method of claim 45, wherein the two cells differ in the activity of said polypeptide.

48. A method of diagnosing fungal, preferably Aspergillus fumigatus, infection comprising the steps of
a. providing a sample from an individual,
b. contacting said sample with an indicator moiety capable of specifically recognising and binding the polypeptide of SEQ ID NO:36, and
c. determining whether a signal has been generated by the indicator moiety.

49. The method of the preceding claim, wherein said indicator moiety is or comprises an antibody, such as an antibody as defined in any of claims 1-22 .

50. A kit for the detection of fungal material, preferably intact fungal cells, most preferably intact Aspergillus fumigatus cells, in a biological sample comprising
a. an indicator moiety capable of specifically recognising and binding the polypeptide of SEQ ID NO:36, and

b. one or more of: a buffer for promoting binding of the indicator moiety to the fungal material; a reagent for generating a detectable signal; and written instructions to the user.

51. The kit of claim 50, wherein said indicator is or comprises an antibody, such as an antibody as defined in any of claims 1-22.